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Studies on the Tumor Initiating Tumor Promoting, and Tumor Co-Initiating Properties of Respiratory Carcinogens

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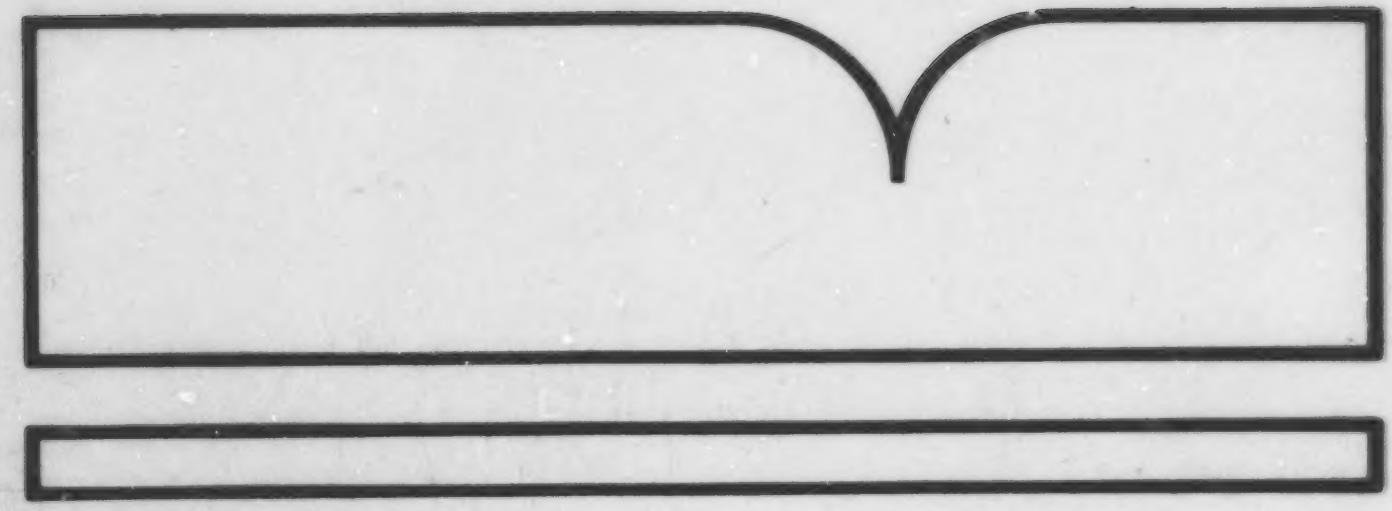

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STUDIES ON THE TUMOR INITIATING, TUMOR PROMOTING, AND TUMOR CO-INITIATING PROPERTIES OF RESPIRATORY CARCINOGENS

by

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16. ABSTRACT

Coke oven emissions, roofing tar emissions, and cigarette smoke represent three diverse human respiratory carcinogens for which good epidemiological information is available. Although the carcinogenic effects of these emissions in experimental animals have been established, the tumor promoting and cocarcinogenic effects have been less well studied, especially those of coke oven and roofing tar emissions.

The tumor promoting effects of a coke oven sample and a roofing tar emission sample were investigated by using SENCAR mouse skin as the experimental test system. The coke oven sample was approximately 12 times as active as the roofing tar sample as a tumor promoter in mouse skin initiated with benzo[a]pyrene (B[a]P). SENCAR mice initiated with B[a]P and promoted weekly with the coke oven sample produced fewer papillomas (or carcinomas) than mice receiving equivalent weekly doses of the coke oven sample alone. The inverse was observed with the roofing tar sample: mice initiated with B[a]P and promoted weekly with the roofing tar sample produced more papillomas than mice treated with equivalent weekly doses of the roofing tar sample alone.

Cocarcinogenesis was studied in SENCAR mice by using the tumor co-initiation protocol. Mice initiated with B[a]P and pyrene and subsequently promoted weekly with 12-*O*-tetradecanoyl-phorbol-13-acetate (TPA) produced more papillomas than mice initiated with B[a]P and promoted with TPA. A roofing tar sample and cigarette smoke condensate each produced synergistic effects with B[a]P on TPA-promoted SENCAR mouse skin, and an organic extract of diesel particulate emissions and B[a]P had attenuative effects. These results are discussed in light of chemical fractionation and chemical identification of some of the components of the samples.

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Studies on the Tumor Initiating, Tumor Promoting, and Tumor Co-Initiating Properties of Respiratory Carcinogens

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We have been interested for several years in studying the tumorigenic and carcinogenic properties of respiratory carcinogens, with major emphasis on complex environmental mixtures (10-13). With mouse skin as the bioassay model, the multifaceted behavior of these chemicals and mixtures can be elucidated using the protocols of tumor initiation, tumor promotion, tumor co-initiation, and complete carcinogenesis. Reviews of mouse skin carcinogenesis have stressed the multistage nature of this process (15,16), the qualitative and quantitative relationships between tumor initiation and complete carcinogenesis, chemical, strain and tissue specificity towards the tumor initiating and tumor promoting properties of chemicals, and biochemical and biological properties related to tumor promotion and progression (15,16). In chemical-based reviews of mouse skin tumorigenesis, it is clear that although a multitude of functionally different chemicals have been examined, the major portion of the data base is comprised of studies relating to polycyclic aromatic hydrocarbons (PAH) and their derivatives (10,14). Studies with complex mixtures also provide a major data base in the mouse skin system, as this tissue is unusually responsive to the tumorigenic and carcinogenic effects of combustion products (11,13,14).

In an earlier review of mouse skin tumorigenesis, we suggested that further evaluation should be undertaken to expand the data base on the tumor-initiating effects of respiratory carcinogens, using several well-known animal and human respiratory carcinogens to probe the

sensitivity of mouse skin as an assay to detect these chemicals (10). The first part of this report describes the results of these experiments.

In addition to studies with individual respiratory carcinogens, we have been evaluating the tumorigenic and carcinogenic effects of complex environmental mixtures. One of the goals of this research program is to compare the potency of human respiratory carcinogens based on epidemiological data with the bioassay potency data obtained from mouse skin tumor initiation and mouse skin complete carcinogenesis experiments as well as the bioassay potency data obtained from short-term genetic toxicology bioassays. Three human respiratory carcinogens were used: emissions from coke ovens, emissions from roofing tar pots, and cigarette smoke. When the potency obtained from mouse skin tumor initiation experiments of these materials was compared with the potency based on human respiratory cancer, a surprisingly close correlation was observed. Over a potency range of 1 to 1,000, the relative incidence of human lung tumors correlated well with the incidence of mouse skin papillomas and carcinomas (1,8). Due to these unique findings, additional experiments were undertaken to evaluate the tumor-promoting and tumor-co-initiating properties of these human respiratory carcinogens. The remainder of this report will describe the results of these studies.

MATERIALS AND METHODS

Sample Generation and Collection

The details of the sample preparation have been reported elsewhere (11,13). The diesel sample was obtained from a 1973 preproduction Nissan-Datsun 220C and represents a dichloromethane extract of diesel particulates collected on a Pallflex fiber glass filter. The coke oven sample is a dichloromethane extract of coke oven material collected from a separator located between the gas collector and the primary coolers at a coke oven battery at Republic Steel, Gadsden, AL. In previous reports, this sample has been referred to as the coke oven main sample (12,13). The roofing tar sample is a dichloromethane extract of roofing tar emissions produced by heating coal tar based pitch at 182-193° in a conventional tar pot and collecting the emissions in a Teflon sock in a bag-house. The cigarette smoke condensate sample was produced from Kentucky R1 cigarettes utilizing a smoking machine and was collected in an acetone-Dry Ice bath.

Mouse Skin Tumorigenesis

Seven- to nine-week-old SENCAR mice (16) bred at the Oak Ridge National Laboratory were used (80 mice of each sex per treatment group). Animals were housed in plastic cages (10 per cage) under yellow light with hardwood chip bedding, fed Purina chow and water ad libitum, and maintained at 22-23° with 10 changes of air per hour. Only mice in the resting hair growth cycle were used. Unless otherwise noted, all treatments were performed using 200 µl of spectral-grade acetone.

Under the tumor initiation protocol, mice were treated with a single topical administration of the test agent, unless otherwise noted. One week after treatment, mice were treated topically, twice weekly with 2 µg of 12-O-tetradecanoylphorbol-13-acetate (TPA). Under the tumor promotion protocol, mice were treated with benzo(a)pyrene (B(a)P) (50.5 µg/mouse) and after one week were treated weekly with the test agents, except for the 4,000-µg/mouse dose that was administered as two weekly doses of 2,000 µg/mouse. Under the tumor co-initiation protocol, mice were treated with both B(a)P (50.5 µg/mouse) and test agent and were subsequently treated twice weekly with TPA (2 µg/mouse).

Skin tumor formation was recorded weekly. Papillomas >2 mm in diameter and carcinomas were included in the cumulative total if they persisted one week or longer. The tumors were verified by standard histological methods.

Survival was >90% in the tumor co-initiation experiments and in the tumor promotion experiments up to week 31.

RESULTS

Tumor Initiation Studies

Using the mouse skin tumor initiation protocol, the potential tumor-initiating activities of four suspect human respiratory carcinogens were evaluated: the metal salts beryllium sulfate, nickel sulfate, and potassium dichromate and 4,4'-methylene bis(2-chloroaniline) (MOCA). In order to maximize the effects of the metal salts, they were administered to the mice by intraperitoneal (i.p.) injection in saline. The mice were subsequently dermally treated twice weekly with TPA for 26 weeks. Except for potassium dichromate, there was no overt toxicity due to treatment with the metal salts. The highest

dose chosen for each was approximately 1/5 of the LD_{50}. Potassium dichromate was completely lethal to these mice at 5,000 µg/mouse. Over a dose range of 0.01-10.0 µg/mouse (beryllium sulfate, Table 1); 0.1-400 µg/mouse (nickel sulfate, Table 2); or 50-1,000 µg/mouse (potassium dichromate, Table 3), none of these chemicals induced significant numbers of mouse skin papillomas. MOCA was administered by dermal application to the dorsal area of mice; these mice were subsequently treated twice weekly with TPA. Over a dose range of 100-200,000 µg/mouse, MOCA did not induce significant numbers of mouse skin papillomas (Table 4).

Tumor Promotion Studies

In order to compare the tumor-promoting activity of the complex mixtures, coke oven and roofing tar, an experiment was performed in which these materials were applied to SENCAR mice previously initiated with 50.5 µg of B(a)P. Over a dose range of 100-5,000 µg/mouse, coke oven and roofing tar were applied weekly to groups of 40 male and 40 female B(a)P-initiated mice. The mice were scored weekly for the appearance of papillomas and squamous cell carcinomas. The data for combined sexes from the 27th week of the experiment are presented in Fig. 1. On a mass basis, the coke oven sample was approximately four times as active as a tumor promoter as the roofing tar sample. Comparison of these results with those obtained from mice initiated with 50.5 µg of B(a)P and promoted with B(a)P (12.6-252 µg/wk/mouse) indicates that on a mass basis, B(a)P is 15.9 times as active as coke oven and 80 times as active as roofing tar.

A comparison was performed between mice treated once with B(a)P and subsequently treated weekly with coke oven or roofing tar and mice treated weekly only with coke oven or roofing tar. Data are presented in Fig. 2 for the coke oven studies when scoring was performed for squamous cell carcinoma formation at week 37. Over a dose range of 1,000-4,000 µg/wk/mouse, mice treated with coke oven alone were more susceptible to carcinoma formation than mice treated once with 50.5 µg of B(a)P and subsequently treated weekly with coke oven. Similar increases were seen in the same animals when they were scored at weeks 30 or 31 for papillomas per mouse (Fig. 3) or for the number of mice bearing papillomas (Fig. 4). However, using the same experimental procedures with roofing tar, the inverse effect was seen. Mice treated once with B(a)P and subsequently treated weekly with 1,000-4,000 µg/wk/mouse of roofing tar bore more papillomas (Fig. 5)

TABLE 1. Mouse skin tumor initiation by beryllium sulfate

Dose, µg[a]	Number of mice surviving		Mice bearing papillomas, percent	
	M	F	M	F
0	40	36	10	11
0.01	39	38	8	18
0.10	39	37	8	22
1.0	37	39	0	18
5.0	38	38	5	18
10.0	36	39	8	21
B(a)P, 50.5	38	39	68	74

[a] Intraperitoneal administration. Mice dermally promoted with TPA.

TABLE 2. Mouse skin tumor initiation by nickel sulfate

Dose, µg[a]	Number of mice surviving		Mice bearing papillomas, percent	
	M	F	M	F
0	40	36	10	11
0.1	39	39	13	15
1.0	40	40	5	8
50	39	40	10	3
100	40	39	5	3
400	40	39	5	8
B(a)P, 50.5	39	39	97	95

[a] Intraperitoneal administration Mice dermally promoted with TPA.

TABLE 3. Mouse skin tumor initiation by potassium dichromate

Dose, µg[a]	Number of mice surviving		Mice bearing papillomas, percent	
	M	F	M	F
0	40	36	10	11
50	38	40	18	8
100	40	40	5	8
500	38	41	5	7
1,000	37	31	11	10
B(a)P, 50.5	39	39	97	95

[a] Intraperitoneal administration. Mice dermally promoted with TPA.

TABLE 4. Mouse skin tumor initiation by MOCA

Dose, µg[a]	Number of mice surviving		Mice bearing papillomas, percent	
	M	F	M	F
0	40	36	10	11
100	35	38	6	3
1,000	39	39	23	15
10,000	38	39	5	5
100,000	38	40	16	15
200,000	37	37	14	5
B(a)P, 25.2	39	39	90	94

[a] Dermal administration. Mice dermally promoted with TPA.

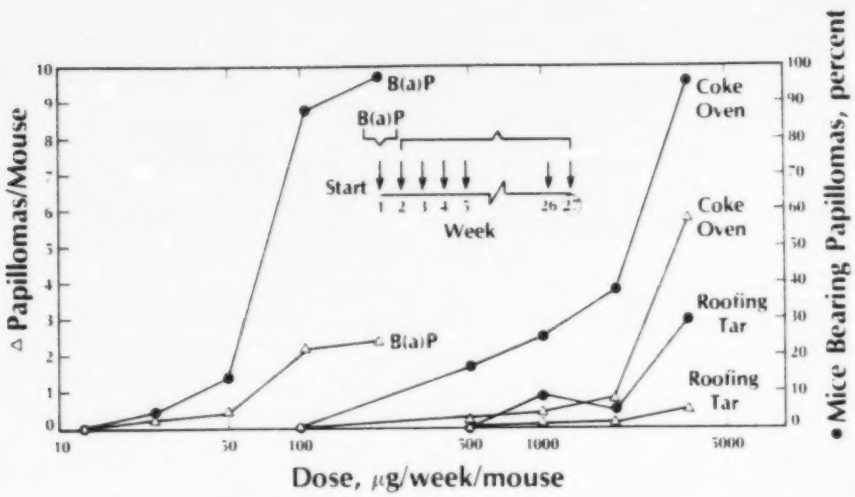

FIG. 1. Comparison of the tumorigenic activities of complex mixtures to B(a)P on B(a)P-initiated SENCAR mouse skin. Benzo(a)pyrene-initiated mice (50.5 μg/mouse) were subsequently treated weekly (except for the 4,000-μg/wk/mouse dose, which was administered as twice weekly doses of 2,000 μg/mouse) with either B(a)P, coke oven, or roofing tar. The circled week number represents the week of scoring.

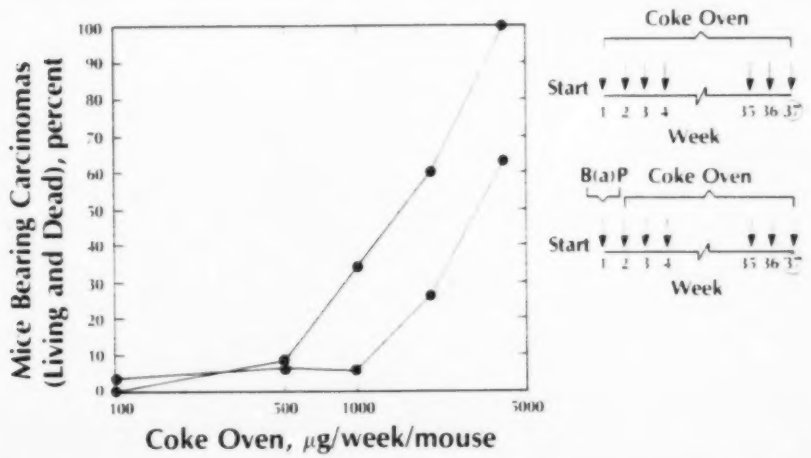

FIG. 2. Formation of squamous cell carcinomas in mice treated weekly with coke oven for 37 wk or treated once with B(a)P (50.5 μg/mouse) and subsequently treated weekly for 36 wk with coke oven. The 4,000-μg/wk/mouse dose was administered as twice weekly doses of 2,000 μg/mouse. Mice were scored on week 37. In B(a)P-initiated mice, the same results were obtained at weeks 37 and 38.

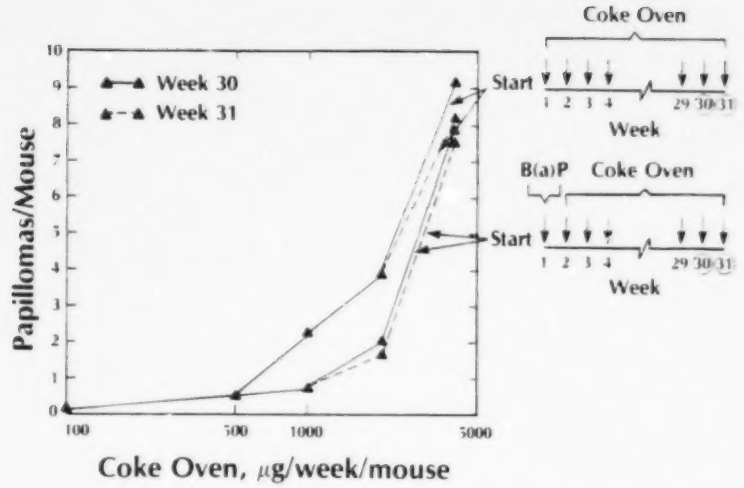

FIG. 3. Skin papilloma multiplicity in mice treated weekly with coke oven for 30 or 31 wk or treated once with B(a)P (50.5 μg/mouse) and subsequently treated weekly with coke oven for 30 or 31 wk. The 4,000-μg/wk/mouse dose was administered as twice weekly doses of 2,000 μg/mouse.

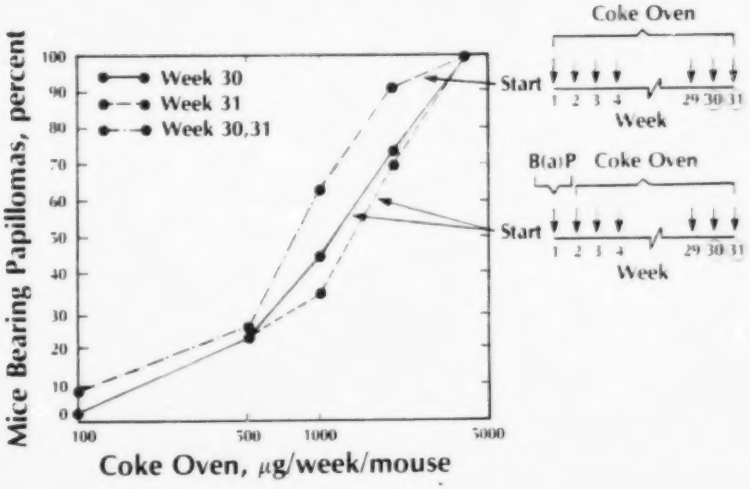

FIG. 4. Skin papilloma incidence in mice treated weekly with coke oven for 30 or 31 wk or treated once with B(a)P (50.5 μg/mouse) and subsequently treated weekly with coke oven for 30 or 31 wk. The 4,000-μg/wk/mouse dose was administered as twice weekly doses of 2,000 μg/mouse.

and had a higher papilloma incidence (Fig. 6) than mice treated weekly only with roofing tar when scored at week 30 or 31 of the experiment.

Tumor Co-Initiation Studies

Tumor co-initiation studies were performed in groups of 40 male and 40 female SENCAR mice by co-administering the test agent with 50.5 µg/mouse of B(a)P, a component of each of the complex mixtures studied. After one week, these mice were treated twice weekly with TPA for up to 30 wk. Papilloma formation was recorded weekly, and the results presented in the following figures are the combination of the results from both the male and female mice.

Experiments were first performed with a PAH known to be found in each of these complex mixtures: pyrene. Using the tumor co-inititiation protocol, pyrene was found to exert a synergistic effect with B(a)P (Fig. 7,8). Pryene is inactive as a tumor initiator at the doses examined; however, when co-administered with B(a)P at 200 or 400 µg/mouse, it produced significant increases in papillomas per mouse (Fig. 7) and smaller increases in papilloma incidence (Fig. 8) compared to mice treated with B(a)P alone. The lower dose of pyrene produced a 50% increase, while the higher dose produced a 100% increase based on tumor multiplicity.

Cigarette smoke condensate was evaluated as a tumor co-initiator and was also found to induce a synergistic effect with B(a)P (Fig. 9 to 11). These effects could only be observed during the early weeks of the studies. Cigarette smoke condensate co-administered with B(a)P at doses of 500-10,000 µg/mouse produced increased papilloma responses compared to B(a)P-treated mice (Fig. 9). Unlike pyrene, however, cigarette smoke condensate did produce a slight tumorigenic response at 1,000 and 2,000 µg/mouse. An examination of the temporal synergistic effects of cigarette smoke condensate with B(a)P at 1,000 and 2,000 µg/mouse with both appropriate controls can be seen in Fig. 10 and 11.

Roofing tar was examined for its ability to induce a synergistic effect with B(a)P as a tumor co-initiator in SENCAR mouse skin. The effects of roofing tar in combination with B(a)P are shown in Fig. 12 to 15. In terms of papilloma incidence, roofing tar exhibited a dose-related effect from 1,000-10,000 µg/mouse in SENCAR mice co-initiated with 50.5 µg B(a)P/mouse (Fig. 12). Unlike pyrene and like cigarette smoke condensate, roofing tar did produce a tumorigenic effect when administered alone to mice as a tumor initiator. At 2,000 µg

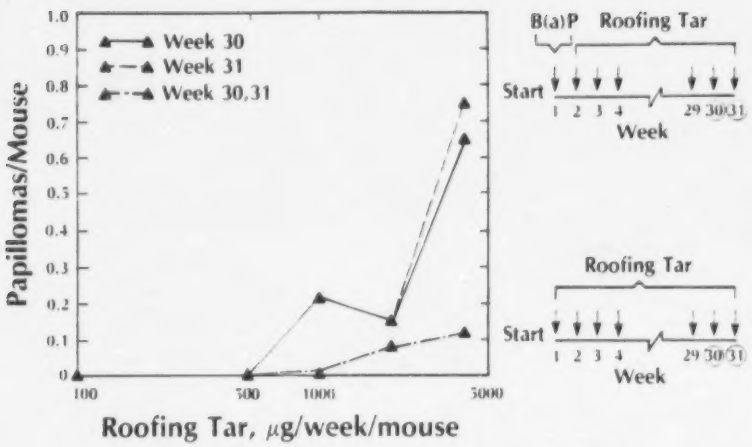

FIG. 5. Skin papilloma multiplicity in mice treated weekly with roofing tar for 30 or 31 wk or treated once with B(a)P (50.5 μg/mouse) and subsequently treated weekly with roofing tar for 30 or 31 wk. The 4,000-μg/wk/mouse dose was administered as twice weekly doses of 2,000 μg/mouse.

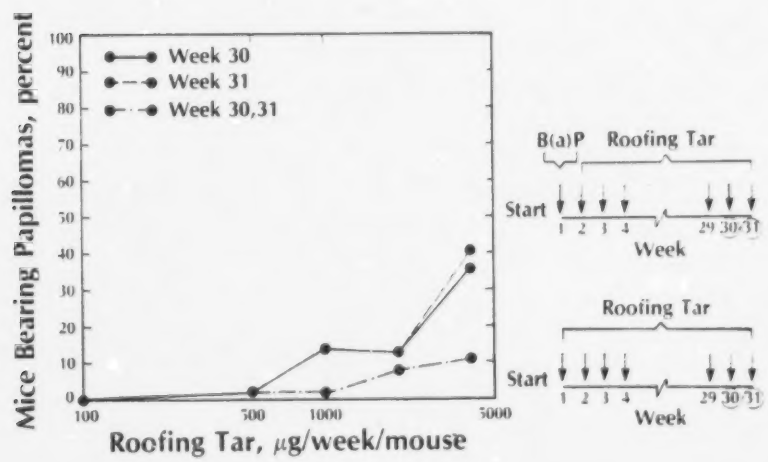

FIG. 6. Skin papilloma incidence in mice treated weekly with roofing tar for 30 or 31 wk or treated once with B(a)P (50.5 μg/mouse) and subsequently treated weekly with roofing tar for 30 or 31 wk. The 4,000-μg/wk/mouse dose was administered as twice weekly doses of 2,000 μg/mouse.

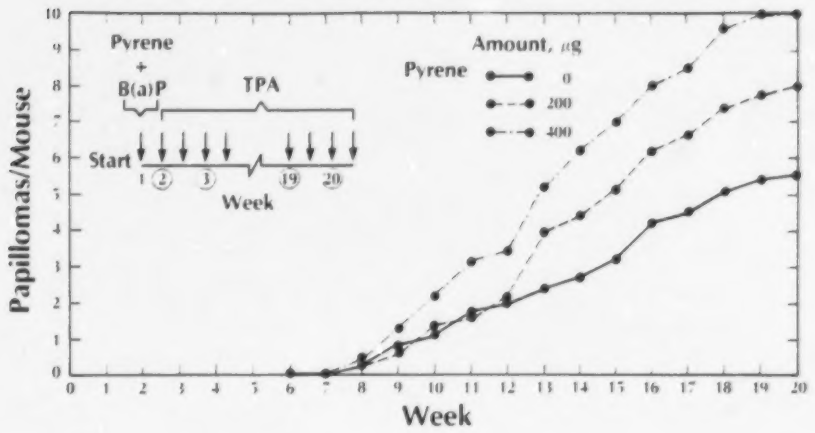

FIG. 7. Synergistic effects of pyrene and B(a)P in SENCAR mice co-initiated with these PAH and promoted with TPA. Data reported as papillomas per mouse. Pyrene has no effect as a tumor initiator at these doses.

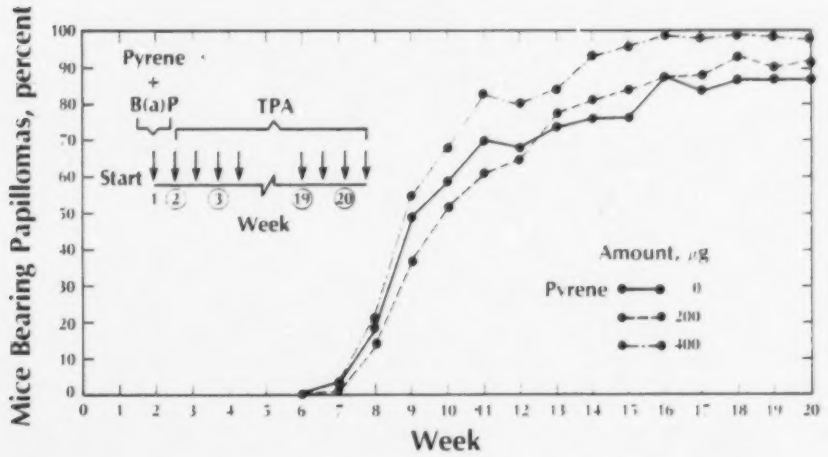

FIG. 8. Synergistic effects of pyrene and B(a)P in SENCAR mice co-initiated with these PAH and promoted with TPA. Data reported as percent of mice bearing papillomas. Pyrene has no effect as a tumor initiator at these doses.

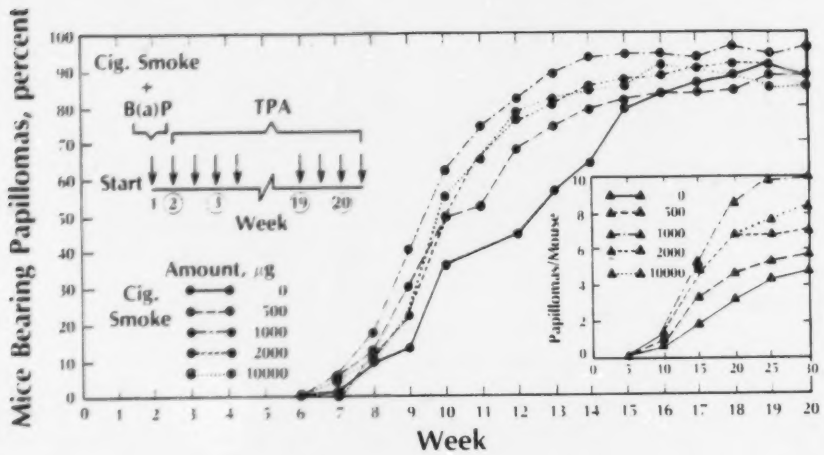

FIG. 9. Mouse skin tumor co-initiation studies with cigarette smoke condensate and B(a)P. Mice were co-initiated with these two agents and subsequently promoted with TPA.

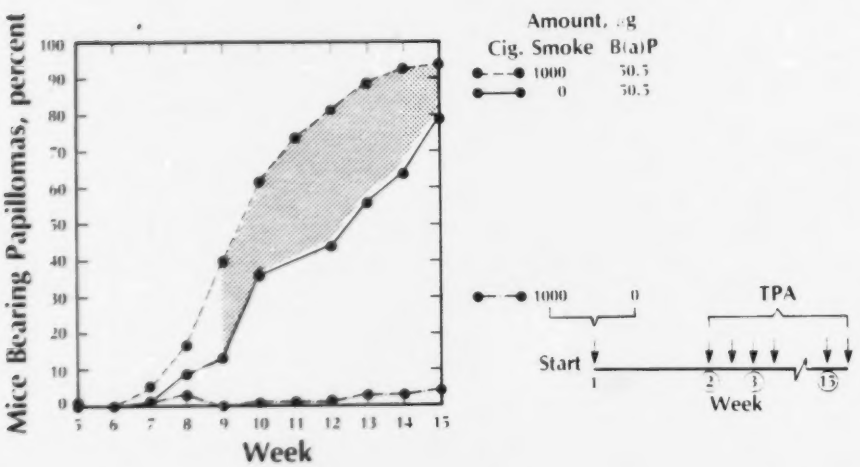

FIG. 10. Temporal synergistic effects of cigarette smoke condensate and B(a)P in the tumor co-initiation protocol. Mice were treated once with cigarette smoke condensate (1,000 µg/mouse) or B(a)P (50.5 µg/mouse) or were treated once with a combination of cigarette smoke condensate and B(a)P. All mice were promoted with TPA.

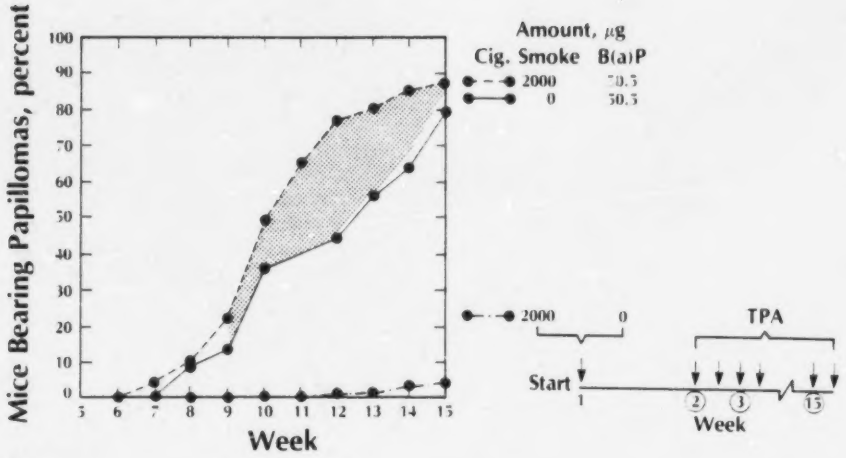

FIG. 11. Temporal synergistic effects of cigarette smoke condensate and B(a)P in the tumor co-initiation protocol. Mice were treated once with cigarette smoke condensate (2,000 µg/mouse) or B(a)P (50.5 µg/mouse) or were treated once with a combination of cigarette smoke condensate and B(a)P. All mice were promoted with TPA.

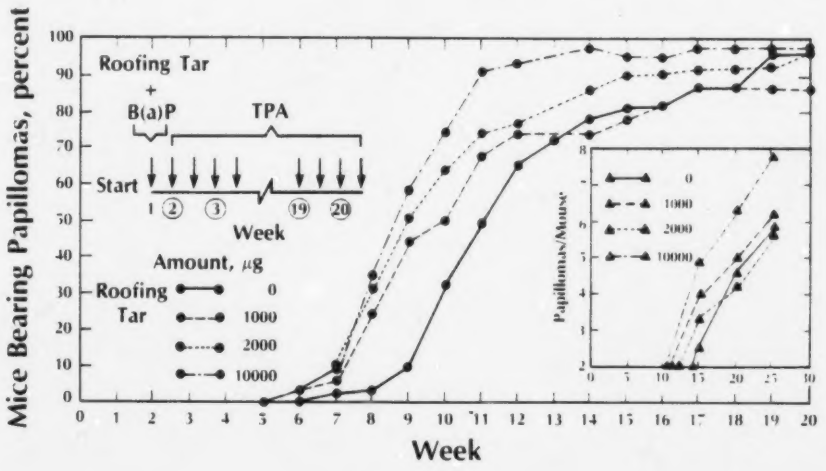

FIG. 12. Mouse skin tumor co-initiation studies with roofing tar and B(a)P. Mice were co-initiated with these two agents and subsequently promoted with TPA.

roofing tar/mouse with B(a)P, a synergistic effect was observed at weeks 8-10 of the experiment (Fig. 13); at later time points, this effect was reduced. Similar effects were measured at the 10,000-µg/mouse dose level for both papilloma incidence (Fig. 14) and multiplicity (Fig. 15).

Diesel was examined for possible synergistic effects with B(a)P on SENCAR mouse skin as a tumor co-initiator (Fig. 16,17). When co-administered with 50.5 µg B(a)P/mouse, diesel at 2,000-10,000 µg/mouse had no demonstrable effect (Fig. 16). However, when considering that both B(a)P and diesel (10,000 µg/mouse) can induce significant tumor-initiating effects by themselves, as indicated in Fig. 17, the fact that their combined effects are not additive indicate that diesel and B(a)P are antagonistic as tumor co-initiators in SENCAR mice.

DISCUSSION

The ability of mouse skin to identify carcinogens that act on respiratory tract tissue is of importance, as this system is commonly used to investigate the activity of complex environmental mixtures isolated from airborne emissions. Extrapolations of the results from mouse skin experimentation to the human respiratory situation are strengthened by the findings that human and animal respiratory carcinogens are also active on mouse skin. It has recently been shown that the relative potency of three complex mixture human respiratory carcinogens obtained from mouse skin tumor initiation studies is approximately equal to their relative potency as human carcinogens (1,8). The studies presented herein were performed to explore the sensitivity of the mouse skin system to known human and animal respiratory carcinogens as single agents. The three metal salts examined were negative at the doses utilized using the i.p. route of administration. MOCA was also not active as a mouse skin tumor initiator when topically administered. A summary of the known human and animal respiratory carcinogens and their activity in mouse skin is presented in Table 5. Of the 11 chemicals or mixtures evaluated in the mouse skin system, 7 were positive. Of the four inactive agents, three were metal salts. These findings suggest that mouse skin can be used to evaluate complex mixtures for carcinogenic activity, especially if they contain PAH. However, mouse skin should not be used to evaluate metal salts or mixtures that contain sufficient quantities of metal salts.

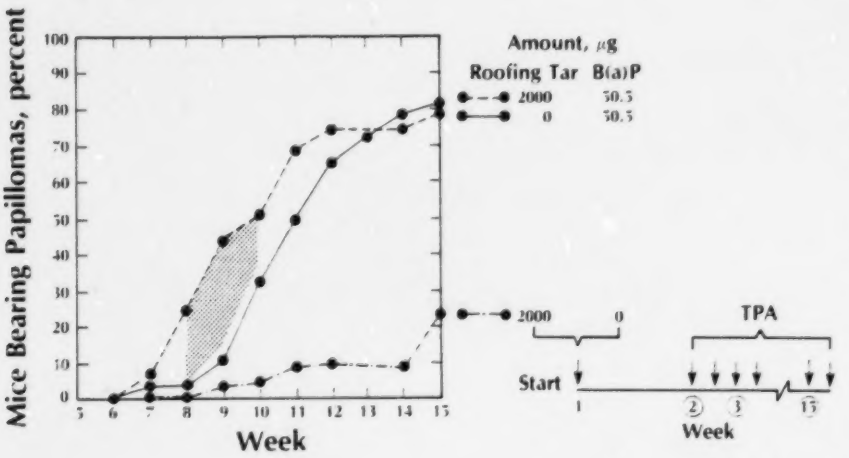

FIG. 13. Temporal synergistic effects of roofing tar and B(a)P in the tumor co-initiation protocol. Mice were treated once with roofing tar (2,000 µg/mouse) or B(a)P (50.5 µg/mouse) or were treated once with a combination of roofing tar and B(a)P. All mice were promoted with TPA.

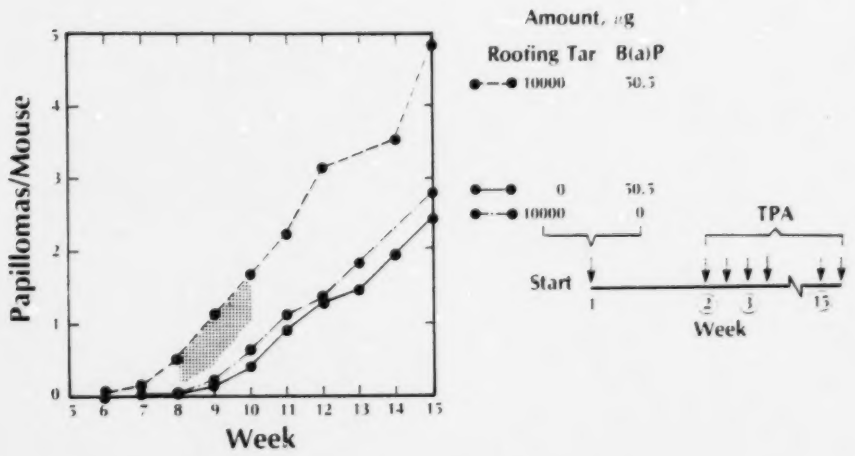

FIG. 14. Temporal synergistic effects of roofing tar and B(a)P in the tumor co-initiation protocol. Mice were treated once with roofing tar (10,000 µg/mouse) or B(a)P (50.5 µg/mouse) or were treated once with a combination of roofing tar and B(a)P. All mice were promoted with TPA. Data are reported as papillomas per mouse.

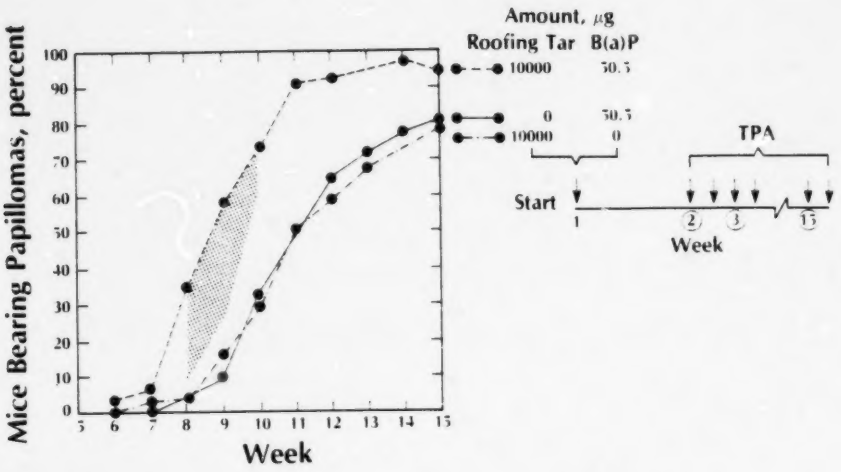

FIG. 15. Temporal synergistic effects of roofing tar and B(a)P in the tumor co-initiation protocol. Mice were treated once with roofing tar (10,000 μg/mouse) or B(a)P (50.5 μg/mouse) or were treated once with a combination of roofing tar and B(a)P. All mice were promoted with TPA. Data are reported as percent of the mice bearing papillomas.

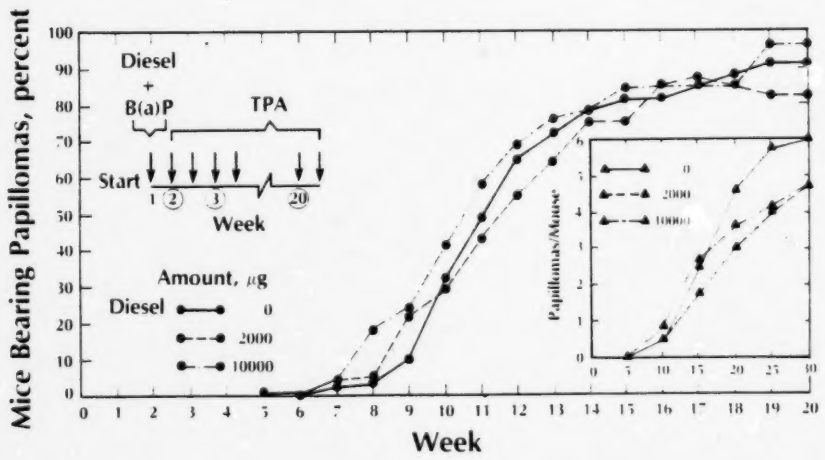

FIG. 16. Mouse skin tumor co-initiation studies with diesel and B(a)P. Mice were co-initiated with these two agents and subsequently promoted with TPA.

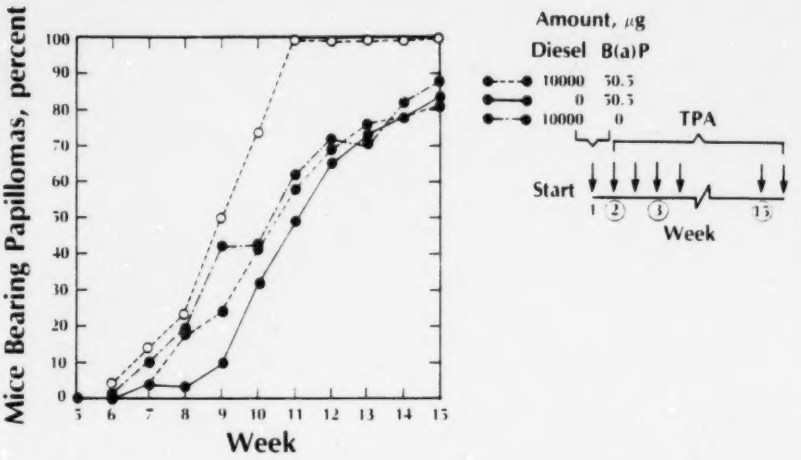

FIG. 17. Temporal potential antagonistic effects of diesel and B(a)P in the tumor co-initiation protocol. Mice were treated once with diesel (10,000 μg/mouse) or B(a)P (50.5 μg/mouse) or were treated once with a combination of diesel and B(a)P. All mice were promoted with TPA. Data are reported as percent of the mice bearing papillomas. The line connecting the open circles represents the expected additive effects of both diesel and B(a)P.

A tumor promoter is defined as an agent that is capable of enhancing the tumorigenic effects of other chemicals and that has for the most part no tumorigenic activity by itself. In general, tumor promoters also have the characteristics of being epigenetic in mechanism and inducing reversible changes, at least in the early stages of their action (15,17). Since we have previously determined that coke oven and roofing tar are complete carcinogens on SENCAR mouse skin, they can be considered to possess both tumor-initiating and tumor-promoting activities (12,13). However, the experimental verification of these conclusions is difficult to establish using the standard tumor initiation/tumor promotion protocol, since they are tumorigenic agents. One of the hallmarks of mouse skin tumor promotion is the early formation of large numbers of papillomas that begin to partially regress after 30 weeks of TPA treatment. Large numbers of papillomas per mouse were observed in coke oven-promoted, B(a)P-initiated mice (Fig. 3), providing direct experimental evidence for the tumor-promoting activity of coke oven. Similar effects were not observed with roofing tar. As a result, the results obtained from the experiments using the tumor promotion protocol can be thought of as sequential syncarcinogenesis, as described by Williams (17).

TABLE 5. Response of respiratory carcinogens in the mouse skin tumorigenesis bioassay system

Chemical	Occupational respiratory carcinogen[a]	Animal respiratory carcinogen[a]	Mouse skin tumorigen[b]
Arsenic	+		
Asbestos	+	+	
Beryllium	+	+	– ?[c]
Carbamates		+	+
Chloromethyl ethers	+	+	+
Chromium	+		– ?[c]
Cigarette smoke	+	+	+[d]
Coke oven	+		+[d]
Isopropyl oil	+		
MOCA	+	+	– ?[c]
Mustard gas	+	+	
Nickel	+	+	– ?[c]
Nitrosamines		+	
PAH	+	+	+
Roofing tar	+		+[d]
Quinolines		+	+
Vinyl chloride	+	+	

[a] Data from (1,3).

[b] Data from (9,14). Includes both tumor initiation and complete carcinogenesis studies.

[c] Based on data presented in this article and limited to the doses employed and the routes of administration utilized.

[d] Data from (13).

The anticarcinogenic effect in mice treated once with B(a)P and then treated weekly with coke oven compared to mice treated weekly only with coke oven, and the syncarcinogenic effect observed with roofing tar, may be explained in terms of DNA adducts. It is reasonable to assume that there are a finite number of sites on the DNA in which PAH-activated species can interact. It is also assumed that the first treatment the mice receive saturates or nearly saturates these potential sites on the DNA. Therefore, the resultant effects of these treatments in terms of tumors is reflected in the efficiency or potency of the adducts in producing tumors. In the case of coke oven, it would seem that adducts produced by coke oven are more productive towards tumor formation than adducts produced by B(a)P. Therefore, mice first treated with B(a)P and then treated with coke oven will exhibit fewer tumors than mice treated only with coke oven. The same argument can be made for roofing tar, in which adducts produced by roofing tar are

less efficient or potent in producing tumors than B(a)P. Therefore, mice first treated with B(a)P and then treated with roofing tar will exhibit more tumors than mice treated only with roofing tar. The content of B(a)P in each of these mixtures (1380 ppm, coke oven; 889 ppm, roofing tar) does not contribute to the tumorigenic activities observed in these experiments (1).

The synergistic effects observed with cigarette smoke condensate and roofing tar are in concert with the determination of chemicals in these samples that are known to exert these activities. 1-Methylindole, 9-ethylcarbazole, and catechol have previously been identified as enhancers of mouse skin tumorigenesis and are present in cigarette smoke condensate (6,7). Pyrene is present in both cigarette smoke condensate and roofing tar (J. Lewtas, personal communication) and produces synergistic effects with B(a)P (Fig. 7,8). In addition to pyrene, roofing tar contains carbazole and methylcarbazoles, both potential cocarcinogens (J. Lewtas, personal communication).

The anticarcinogenic effects observed with combined treatment of mice with diesel and B(a)P can be explained by alterations in the metabolism/detoxification of B(a)P that is induced by diesel. Haugen and Peak (5) have reported that fractions from coal-derived oil suppressed the rat liver S9 metabolism, covalent DNA binding, and bacterial mutagenic activity of B(a)P.

It is clear from this work and the work of others that B(a)P content cannot account for all of or even a major portion of the tumorigenic activity of coke oven, roofing tar, or diesel. Chemical class fractionation of these mixtures and subsequent bioassay of the fractions in *Salmonella typhimurium* for mutagenic activity has shown that fractions containing the mutagenic activity are not the fractions that contain the greatest mass. More importantly, for each complex mixture, the fraction that contained B(a)P had $<22\%$ of the total mutagenic activity of the unfractionated mixture (2). From tumor initiation studies, B(a)P can account for up to approximately 30% of the tumorigenic activity of coke oven, roofing tar, or diesel (Fig. 18). Grimmer et al. (4) have reported that B(a)P accounts for only 2.4% of the total carcinogenicity of automobile exhaust condensate. Therefore, other carcinogens, cocarcinogens, and syncarcinogens must play a major role in the carcinogenic activity of these complex environmental mixtures.

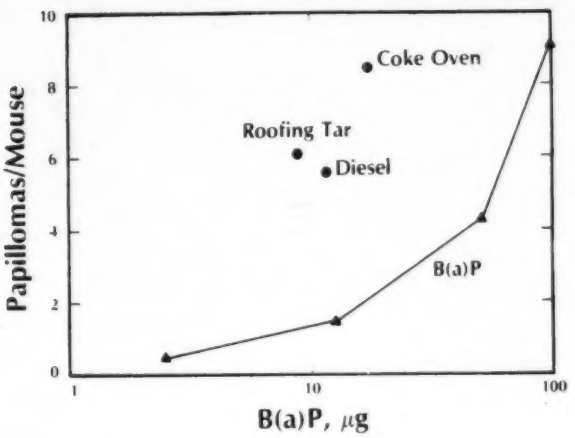

FIG. 18. Mouse skin tumor initiation: relationship between B(a)P content and tumorigenic effects. SENCAR mice were initiated with 10 mg of each complex mixture and promoted with TPA. For the B(a)P data, mice were initiated with various doses of B(a)P and promoted with TPA.

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Running Title: Tumor Initiating, Promoting, and Co-Initiating Properties